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NAME OF AUTHOR/*NOM DE L'AUTEUR* Joy Steele

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NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* Dr. K. Pearson.

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Intersegmental Coordination of Walking in the Cockroach, *Periplaneta americana*

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

DEPARTMENT Physiology

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Intersegmental Coordination of Walking in the Cockroach, *Periplaneta americana*

submitted by Joy Steele

in partial fulfilment of the requirements for the degree of Master of Science

P.S. Pearson
Supervisor

A.N. Spence

R.K. Mitchell

M.S. French

Date 23 April 79

To Jim and Olga

ABSTRACT

Rhythmic leg movements during walking in the cockroach have been shown to be generated by a group of ganglionic interneurons collectively termed the flexor burst generator (Pearson & Iles, 1970). In order for intersegmentally coordinated walking to be produced, the flexor burst generator of each leg must receive the correct timing cue so that each leg steps at the correct moment with respect to the other legs. Several possible mechanisms could underlie intersegmental coordination, that is timing cues could be communicated to the flexor burst generators of the individual legs by: 1) central neural pathways between the individual flexor burst generators; 2) intersegmental reflexes; 3) intrasegmental reflexes operating via a mechanical linkage such that the movement of any one limb alters the sensory input onto another single leg flexor burst generator. Evidence for two of the above mechanisms was obtained in this study: 1) mechanical linkage of intrasegmental reflexes and 2) a central neural pathway between the pro- and metathoracic flexor burst generators not involving the mesothoracic flexor burst generator.

Phase relationships between stepping movements of the legs were determined from recordings of electromyograms from ipsilateral flexor leg muscles. For animals walking on a flat surface with their mesothoracic legs restrained to the thorax, the phase of stepping of a prothoracic leg in the cycle of an ipsilateral metathoracic leg was variable with the most frequent phase relationship being around 0.5. In comparison, the phase relationship for normal animals was 1.0.

The animals with their mesothoracic legs restrained were then suspended above mercury where they could walk freely while their bodies were supported. In this situation, a stepping movement of one leg could not affect the load carried by another leg or the position of another leg. A complete loss of coordination between stepping movements of the pro- and metathoracic legs was exhibited by animals placed in this situation. Thus it was shown that it was the mechanical linkage of intrasegmental reflexes that was responsible for the loose 0.5 phase relationship exhibited by animals walking on a flat surface with their mesothoracic legs restrained. It is possible that this mechanism of intersegmental coordination could play a role in normal walking especially when the animal walks over uneven terrain.

Previous investigations (Pearson & Iles, 1973) led to the suggestion that the central neural coordinating mechanisms may involve a pathway between the pro- and metathoracic flexor burst generators via the mesothoracic flexor burst generators. Evidence obtained in this study suggests that there may also be a central pathway between the pro- and metathoracic flexor burst generators <u>not</u> involving the mesothoracic flexor burst generators. In experiments on mesothoracic amputees, activity in the mesothoracic flexor burst generator did not seem to influence the phase relationships between stepping of the pro- and metathoracic legs. When the legs were amputated proximal to the trochanter, no distinct mesothoracic flexor bursts were recorded during walking. However, if the legs were amputated distal to the trochanter, two mesothoracic flexor bursts per step cycle were recorded. Despite the differences in mesothoracic flexor burst

activity, the phase relationships between the pro- and metathoracic legs was the same in the two different types of amputees. This suggests that the pro- and metathoracic flexor burst generators are coupled by a central neural pathway not involving the mesothoracic flexor burst generator. A model describing the central and reflex pathways involved in intersegmental coordination is presented.

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INTRODUCTION

Animals execute a variety of behaviors during which movements of a number of body parts are kept in a constant time relationship with one another. Such precise coordination of body parts is exhibited during the performance of various rhythmic behaviors: ventilation, stridulation, feeding and locomotion. Considerable progress has been made during the past few decades in characterizing the neural mechanisms underlying coordination of movements during rhythmic behaviors in invertebrates.

One such behavior in which the coordination of timing of limb movements is crucial is locomotion. In order to understand how individual appendages achieve coordination during locomotion, attempts have been made to characterize the role played by central neural elements and the role played by sensory feedback. The relative importance of the central and peripheral control mechanisms has been found to vary with the motor act being performed and with the environmental constraints imposed upon the animal.

Evidence has been obtained which demonstrates that during locomotion the movement of each limb is generated centrally by what is usually referred to as a central oscillator (Stein, 1971; Pearson and Iles, 1973; Shik and Orlovsky, 1976). In order to produce coordinated locomotion, it is necessary for the central oscillators to be coupled in some way. Theoretically there are at least three ways this coupling could be achieved: 1) direct coupling between the different oscillators by a pathway entirely within the central nervous system (central coupling); 2) direct reflex pathways from one leg

onto the oscillator of other legs (intersegmental reflexes); 3) a movement of one leg changing the position of other legs and the load carried by other legs and therefore altering the activity in intrasegmental reflex pathways onto the oscillators in the other legs (mechanical linkage of intrasegmental reflexes).

The role played by proprioceptive feedback and by central neural mechanisms in the coordination of timing of movements of different body parts has been investigated in a number of different species of invertebrates such as swimmeret beating in the crayfish and walking in insects. Before describing the aims of the present investigation, a brief review of each of these systems is given in order to illustrate some of the mechanisms employed for coordinating activity in different body segments.

Crayfish Swimmeret System

The neural basis of intersegmental coordination of appendage movement has been investigated in the swimmeret system of the crayfish. Ikeda and Wiersma (1964) initially demonstrated that the isolated abdominal ganglia of the crayfish can produce intersegmentally coordinated sequences of swimmeret motor output. Therefore, the basic pattern of motor output does not depend on sensory input. The central neural mechanisms of intersegmental coordination were investigated further and neurons were located in the interganglionic connectives which were active during the discharge of specific swimmeret motoneurons (Hughes and Wiersma, 1960). It was suggested that these cells could be influential in coupling the movements of the swimmerets.

Work on the crayfish system was extended by Stein (1971, 1976). Stein demonstrated that intersegmental coordination of swimmeret movement is not accomplished by information carried in the command neurons (neurons capable of eliciting an entire behavior) since intersegmental coordination of rhythmic motoneuron output persisted after the command neurons were severed. When the remaining portion of the connective was cut, coordination of intersegmental motor output was completely lost. Coordinating neurons were found in this part of the connective and bursts of activity of these interneurons was shown to be essential for intersegmental ordering of motor output (Stein, 1971).

The properties of the coordinating neurons were then examined. It was demonstrated that selective stimulation of the coordinating neurons could alter the phase of motor output from an oscillator (Stein, 1971). The phase modulation occurred in response to a single burst of activity in the coordinating neuron. Both phase advances and phase delays could be produced by the stimulus when delivered at different times in the cycle of the second, modulated oscillator.

In summary, interganglionic interneurons in the crayfish abdominal ganglia have been found which appear to be involved in intersegmental coordination of motor output. The coordinating neurons serve to couple the oscillators responsible for generating motor output to individual swimmerets. The coupling signal carried by the coordinating neurons has two characteristics: 1) it contains phase information about the movement state of one oscillator and 2) it can phase modulate the rhythmic motor output of an adjacent oscillator. No

evidence was obtained for the involvement of sensory signals in the coupling of the oscillators. Therefore, work on the crayfish swimmeret system has conclusively demonstrated the existence of central neural elements which are involved in the intersegmental coordination of motor output (central coupling).

Stick Insect Walking System

Although intersegmental coordination has also been examined in walking insects, very little is known about the details of the underlying neural mechanisms. Two insects which have been extensively studied are the stick insect, *Carausius morosus* and the cockroach, *Periplaneta americana*. The walking stick insect system is discussed first and the cockroach system is described later.

A behavioral analysis of the temporal organization of walking movements in first instar and adult stick insects has been done by Graham (1972) and Wendler (1966). The stepping movements exhibited by an insect walking in a straight line on a smooth horizontal surface differ for low and high speeds of progression. At low walking speeds, at least four legs are in contact with the ground at any one time and the legs on each side of the body step with a metachronal rhythm (L3 L2 L1 R3 R2 R1). At high speeds of progression the animal exhibits a tripod gait, i.e., the stepping of ipsilateral pro- and metathoracic legs and contralateral mesothoracic leg alternates with stepping of the remaining three legs. Consequently, when the animal is using the tripod gait, it is supported by three legs at any one time.

Von Buddenbrock (1921) and Wendler (1966) investigated the

effect of amputation of the middle pair of legs. In these amputees, the fore- and hindlegs stepped with a phase relationship of about 0.5 instead of in phase as they do in the normal intact animal. The amputees exhibited the same stepping pattern while walking on a 'treadmill' with their weight supported. Thus, it was concluded that the alteration of stepping pattern brought about by amputation of the middle pair of legs was the result of the new mechanical situation created by amputation. Wendler also showed that an alteration in stepping pattern could be produced by preventing the middle legs from stepping. In these experiments, the middle legs were placed on small platforms which were fixed in relation to the body. The coordination between ipsilateral fore- and hindlegs was changed and quite often resulted in 'gliding coordination' as Wendler (1966) termed it. That is, the stepping frequency of the foreleg was somewhat higher than the hindleg and consequently, the phase of stepping of the foreleg in the cycle of the hindleg was variable. He concluded that the afference from the unmoved middle leg was not sufficient to cause the other four legs to move with their normal coordination and that the phasic afference from the normal movements of the leg seemed to be necessary for normal coordination. Wendler attempted to locate the origin of this essential proprioceptive input by removing various lengths of the legs. Removal of the middle legs above the femur/tibia joint brought about the stepping pattern expected of this sort of amputee. However, if the missing portion of the legs were replaced by prosthetic limbs such that contact with the substrate occurred, then normal coordination was exhibited. According to these results, he

concluded that the receptors of the proximal portion of the leg are sufficient to produce normal coordination insofar as the normal mechanical stimulus is conducted through the leg.

Additional investigation of the walking system of the stick insect has been carried out by Graham (1977a). He examined the step patterns for insects executing turns and for insects initiating walking. In the adult stick insect, Graham observed that change in direction is brought about by differential variation in the step frequency on either side of the body. Thus, it would appear that during the execution of a turn the right and left sides become temporarily uncoupled. Also, on occasion, legs of the same segment were observed to step almost in synchrony upon starting or turning. The right and left sides also appeared to be uncoupled in the nymph (first instar) when it walked using the metachronal gait.

Graham (1977a) carefully examined the stepping patterns of the stick insect with various combinations of legs amputated. In amputees with one leg or any combination of two legs missing, 'coupling' across the body was strong and all the amputees showed well-defined phase relationships between the legs of the same segment in straight walking. The uncoupling or independent operation of the right and left sides was relatively infrequent or occurred only during deliberate turning movements.

On the basis of stepping patterns observed in amputees and in animals with various legs restrained in such a manner that they could not participate in stepping movements, Graham (1977b) proposed a model for the central neural mechanisms underlying the intersegmental

coordination of stepping. In the model, each leg has its own separate burst generator or oscillator. Inhibitory coupling between right and left sides was postulated to occur only in the meso- and metathoracic segments. Thus, a gait in which the prothoracic legs step simultaneously is permissible in the model. Such a gait is used by an amputee with the mesothoracic leg of either side missing. No examples of L3 and R3 stepping simultaneously were found, even in animals with the mesothoracic legs restrained in which there was a general breakdown in the coordination between the legs of the pro- and metathoracic segments. A hierarchy of natural oscillator frequency was assumed with the posterior single leg oscillators having the highest natural frequency. Thus, the posterior single leg oscillators would be expected to be a source of timing for the entire animal. Graham (1977b) used a computer simulation to test his model and was able to simulate complete sequences of behavior, including turns, changes in direction and transitions between different stepping patterns. In the model, cycle-dependent intersegmental delay was achieved entirely by central mechanisms. The simulated step patterns were comparable to the step patterns exhibited by first instar and adult stick insects. However, he did not attempt to use his model to simulate the step patterns exhibited by amputees or by animals with the middle pair of legs restrained.

Cockroach Walking System

The neural control of walking has also been investigated in the cockroach walking system. Cockroaches walking in a straight line on a smooth horizontal surface exhibit the same gaits observed for the

stick insect, i.e., a metachronal gait at low speeds and a tripod gait at high speeds of progression (Delcomyn, 1971a). The tripod gait is maintained over a wide range of walking speeds because as the speed of locomotion increases there is a decrease in the protraction time of all legs and a decrease in the interval between protractions in adjacent ipsilateral legs (Pearson & Iles, 1970).

In the cockroach walking system, the stepping movements are thought to be produced by a group of interneurons which will be referred to as a flexor burst generator. Evidence for the existence of a burst generator comes from experiments where motoneuron activity of flexors and extensors of one segment were recorded (Pearson & Iles, 1970). Flexor burst activity in a completely deafferented preparation could be elicited with or without reciprocal extensor motoneuron activity. Intracellular recordings from the metathoracic ganglion revealed the existence of non-spiking interneurons whose membrane oscillations are in phase with levator motoneuron activity (Pearson & Fourtner, 1975). Experimentally-induced depolarizations of one particular non-spiking interneuron excited the flexor motoneurons. This interneuron appears to be an important element of the burst-generating system. At least four different non-spiking interneurons were physiologically identified in the metathoracic ganglion. However, interneuron I (excites flexor motoneurons) seemed to be the most important one for the generation of rhythmic bursts of activity in the flexor motoneurons. There appears to be only one interneuron I per hemiganglion. Cobalt staining revealed the structure of the cell which was virtually identical in the eight preparations examined.

Interneuron I also appears to inhibit the motoneuron D_s which is a motoneuron partially responsible for producing the extension movement of the leg. Hyperpolarization of interneuron I removed the inhibition but did not bring about high levels of activity in D_s.

It has been demonstrated that the neuronal systems responsible for the generation of flexor bursts (flexor burst generators) in the mesothoracic and metathoracic segments are coupled centrally (Pearson & Iles, 1973). In deafferented preparations, there was a strong tendency for activity in the flexor motoneurons of one leg not to overlap flexor activity of an adjacent leg and vice versa. Thus, it was proposed that the burst-generating systems in each segment mutually inhibit one another, and that the pro- and metathoracic flexor burst generators are coupled to one another by a central neural pathway via the mesothoracic flexor burst generator. In addition, recordings were made from interganglionic neurons which discharged in phase with flexor bursts. These interganglionic neurons may serve to transmit timing cues from one ganglion to the next and may mediate the inhibitory coupling. The possible importance of these interneurons in coordinating leg movements was strengthened by the observation that severing the lateral halves of the meso- and metathoracic connectives which contained the interneurons abolished all locomotory movements of the metathoracic legs. Severance of the medial halves of the connectives did not alter the normal tripod gait.

In this study, attempts were made to investigate further the nature of the central neural coupling of the flexor burst generators. In particular, are the pro- and metathoracic flexor burst generators

coupled 'directly', that is, is there a pathway between them? Or, are they coupled only via the mesothoracic flexor burst generator?

Sensory input from leg receptors also appears to be important in coordinating the stepping movements of the legs in the cockroach. Not all aspects of the burst patterns recorded in deafferented preparations corresponded to those observed in intact walking animals (Pearson & Iles, 1973). The most noticeable difference was that in deafferented animals the mesothoracic burst begins immediately after the end of the metathoracic burst or vice versa, whereas in walking animals, there is an obvious latency which depends on the walking speed. This marked difference indicates that the leg movements are not entirely coordinated centrally and must depend to some extent on sensory input. In addition, amputation of various combinations of legs resulted in an altered stepping pattern. In the case of amputation of the mesothoracic legs, the immediate change in coordination of stepping appears to be the result of loss of afferent input from the mesothoracic legs rather than the result of new mechanical situation (von Buddenbrock, 1921; Hughes, 1957; Wendler, 1966; Delcomyn, 1971b).

Thus, it is of interest to examine the role of sensory input in the coordination of walking in a freely behaving animal. Intersegmental reflexes have been found to be very weak or entirely absent (Pringle, 1940; Wilson, 1965) and in general it seems that they must be of only slight importance in coordinating the legs. On the other hand, intrasegmental reflexes may play an important role in coordinating stepping of the legs. Indeed, cockroaches have extensive

proprioceptive systems which transmit position and loading information for each leg to the central nervous system (Pringle, 1961). For example, the hair plate afferent fibers have been shown to monosynaptically excite extensor motoneurons and to disynaptically inhibit flexor motoneurons (Pearson, Wong and Fourtner, 1976). Thus input from the trochanteral hair plate controls the amplitude of flexion (Wong and Pearson, 1976). The trochanteral campaniform sensilla have been shown to inhibit the flexor burst generator (Pearson and Iles, 1973) and to excite extensor motoneurons (Pringle, 1940; Pearson and Iles, 1973). In addition, there is evidence that compensations for increased load during walking may be achieved by a positive feedback of proprioceptive input from the campaniform sensilla to increase the output of extensor motoneurons during the stance phase in a single limb (Pearson, 1972). On the basis of what is known about the operation of intrasegmental reflexes in a single leg, Pearson and Iles (1973) suggested that intrasegmental reflexes operating via a mechanical linkage may be involved in intersegmental coordination. In other words, it seemed likely that the movement state of one leg would affect the timing of stepping in other legs. However, no evidence was obtained to support this hypothesis. The experiments conducted in this investigation were designed to test the hypothesis that the mechanical linkage of intrasegmental reflexes can serve as an intersegmental coordinating mechanism.

MATERIALS AND METHODS

All experiments were performed on adult male cockroaches, *Periplaneta americana*. To examine intersegmental coordination, electromyograms of the coxal levator muscles (flexors of the femur) of ipsilateral left legs were recorded by implantation of bipolar electrodes. The recording electrodes, which were copper wires 75 µm in diameter insulated except at the tips, were inserted through small holes in the cuticle while the animal was under CO_2 anesthesia. Two recording electrodes were placed in each set of levator muscles. The technique is described more fully by Pearson (1972). The attachment of the electrodes did not alter the movements of the leg to which they were attached in any way that was obvious from visual inspection. The signals were amplified by conventional means and recorded on FM tape for later filming and analysis.

In all legs of these animals, contraction of the posterior coxal levator muscles produces flexion movements of the femur during the protraction phase of the step cycle (Pearson, 1972). For the prothoracic legs, these muscles also contract during the initial part of retraction. Only the prothoracic levator burst associated with flexion of the femur during protraction was used in the determination of phase relationships. The innervation of the levator muscles and their antagonists, the coxal depressor muscles (extensors of the femur) has been described (Pearson and Bergman, 1969; Pearson and Iles, 1970). The metathoracic levator muscles 181 and 182 (notation of Carbonell, 1947) are innervated by nerve 6Br4 (notation of Pipa and Cook, 1959) and the depressor muscles 177D, E, 178 and 179 are innervated by 5r1.

The gross anatomy and neuromuscular organization are very similar in all legs (Carbonell, 1947; Pipa and Cook, 1959). The receptors of the leg have been described by Nijenhuis and Dresden, (1952).

Recordings were made in the following preparations:

1) normal intact animals; 2) animals with their mesothoracic legs tied to the thorax in a flexed position by a fine wire through holes in the tibia and the femur so that the legs could not participate in walking (fig. 1a); 3) animals with their mesothoracic legs amputated distal or proximal to the trochanter (fig. 1b) and 4) animals with their mesothoracic legs tied to the thorax and the trochanteral campaniform sensilla (groups 1 to 5) (fig. 1c) of the mesothoracic legs destroyed. The campaniform sensilla of the trochanter were mechanically destroyed by using a sharp dental drill. Campaniform sensilla occur on the legs in the following positions: four groups on the trochanter, one on the base of the femur on the dorsal side, one on the outside of the base of the tibia (Pringle, 1938).

The animals were then made to walk in one of several of the following situations: 1) smooth horizontal surface (flat piece of plywood); the animals were prevented from moving outside an area two feet in diameter by attaching a fine light thread to an insect pin inserted through the dorsal cuticle of the mesothoracic segment and to an elevated support in the center of this area; 2) upon a pool of elemental mercury held in a Petri dish: the animals were restrained only by 10 cm length of string attached to their dorsal cuticle of the mesothoracic segment. Thus the animals supported their own weight; 3) a pool of mercury with their body supported in such a manner that

Figure 1. a. View of the left side of *Periplaneta americana* Mesothoracic legs were tied to the thorax in a flexed position by passing a fine wire through holes made in the tibia and the femur with an insect pin (location of the holes indicated by dots).

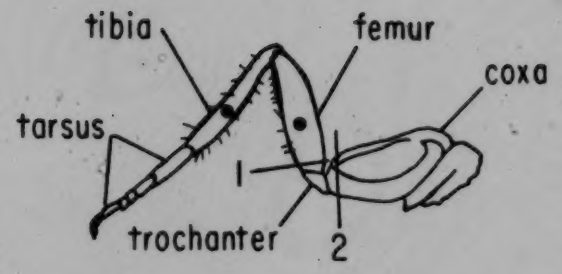
b. The right mesothoracic leg of *Periplaneta americana*. Ventral view showing the levels of amputation. Position 1 is just distal to the trochanter. Position 2 is just proximal to the trochanter. Location of holes used in restraining the mesothoracic legs are shown again.

c. Ventral view of the trochanter of the right mesothoracic leg. Locations of groups 2,3,4 of the campaniform sensilla and the trochanteral hair plate are indicated.

d. Dorsal view of the trochanter indicating the location of groups 1 and 5 of the campaniform sensilla.

a

b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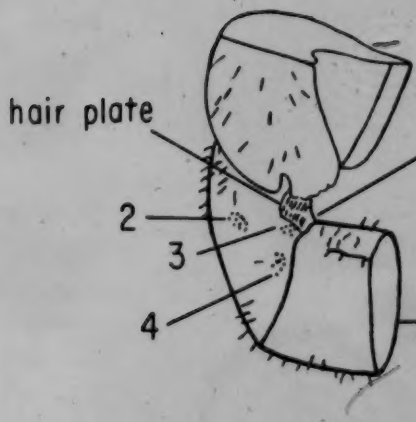
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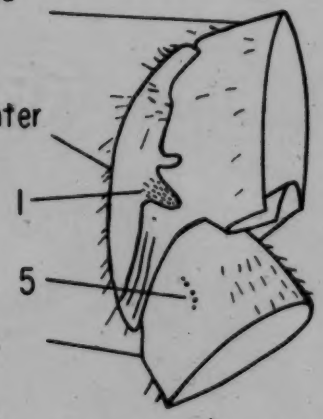
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a stepping movement of one leg could not alter the load carried by other legs or the position of other legs. This was achieved by attaching the dorsal cuticle (mesothoracic segment) of an animal to a small bracket using insect pins. A piece of heavy copper wire formed the base of the bracket to which the animal was attached. A piece of wire, 90° to the base and 2 cm long, extended through a small circular washer attached to a rod which was held by a ring stand. A bead of solder was placed at the top end of the copper wire so that the entire bracket could revolve. This permitted the animal to change directions during walking. The animals were held stationary at a level where all legs just touched the surface of the mercury.

Only the data for walking speeds greater than two steps per second and for walking in a straight line were analyzed. Data on walking speeds less than two steps were included in the analysis of the results from amputees. Thus, only medium to fast walking when then animal normally uses the tripod gait was studied.

The latencies between the occurrence of levator bursts were measured from the filmed data. *Phase* was then calculated by dividing lag time by cycle time. *Lag time* is defined as the time from the beginning of a levator burst of one leg to the beginning of a levator burst in another leg. *Cycle time* is the time from the beginning of a levator burst of one leg to the beginning of the next successive burst in the same leg. Each phase value then describes the timing of stepping between pairs of legs. When the legs step at the same moment, the phase is 0 or 1. When stepping of two legs strictly alternates, the phase is 0.5.

RESULTS

Normal Walking

During walking the timing of movements of the six legs of the cockroach are coordinated in a very precise manner. The resulting stepping patterns have been examined over wide range of stepping frequencies for an animal walking in a straight line on a smooth horizontal surface (Delcomyn, 1971a). When an animal walks slowly with fewer than two steps per second, it exhibits a metachronal stepping pattern (R3 R2 R1 L3 L2 L1). When the stepping frequency is greater than two steps per second, the animal exhibits a tripod gait, i.e., stepping of ipsilateral pro- and metathoracic legs and contralateral mesothoracic leg alternates with stepping in the remaining three legs. Thus, at any one moment, the animal is supported by at least three legs.

Phase relations between stepping of different legs can easily be determined from electromyograms of flexors bursts as illustrated in figure 2. Interappendage phase relations of the tripod gait remain relatively constant over all walking speeds (fig. 3a). Since the phase is independent of cycle time, the data can be plotted as a frequency histogram (fig. 3b). For adjacent ipsilateral legs, the phase is close to 0.5. For ipsilateral pro- and metathoracic legs the phase is close to 1.0.

Amputation of the Mesothoracic Legs

Experiments in which the mesothoracic legs were amputated distal and proximal to the trochanter (fig. 1b) were performed to verify the experimental procedure as these experiments have been done

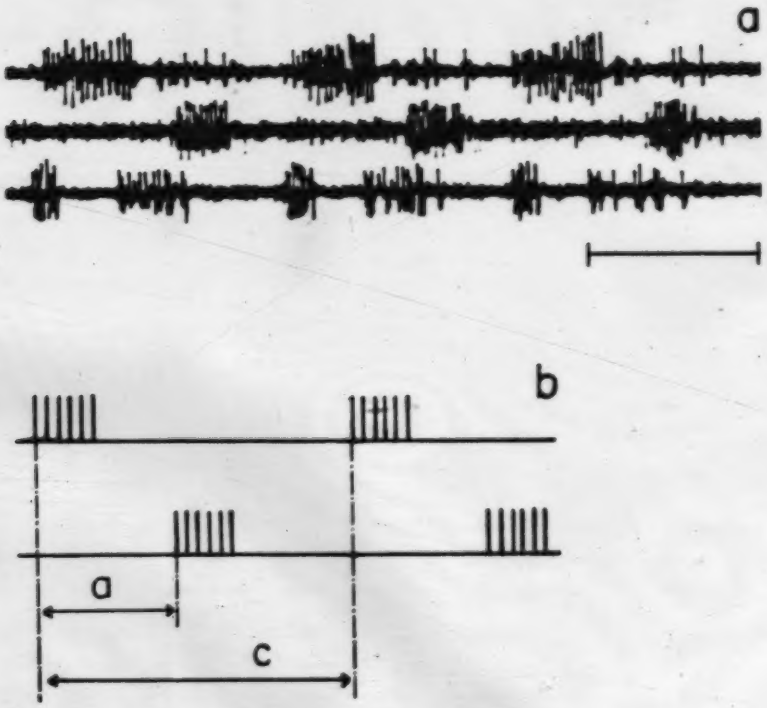
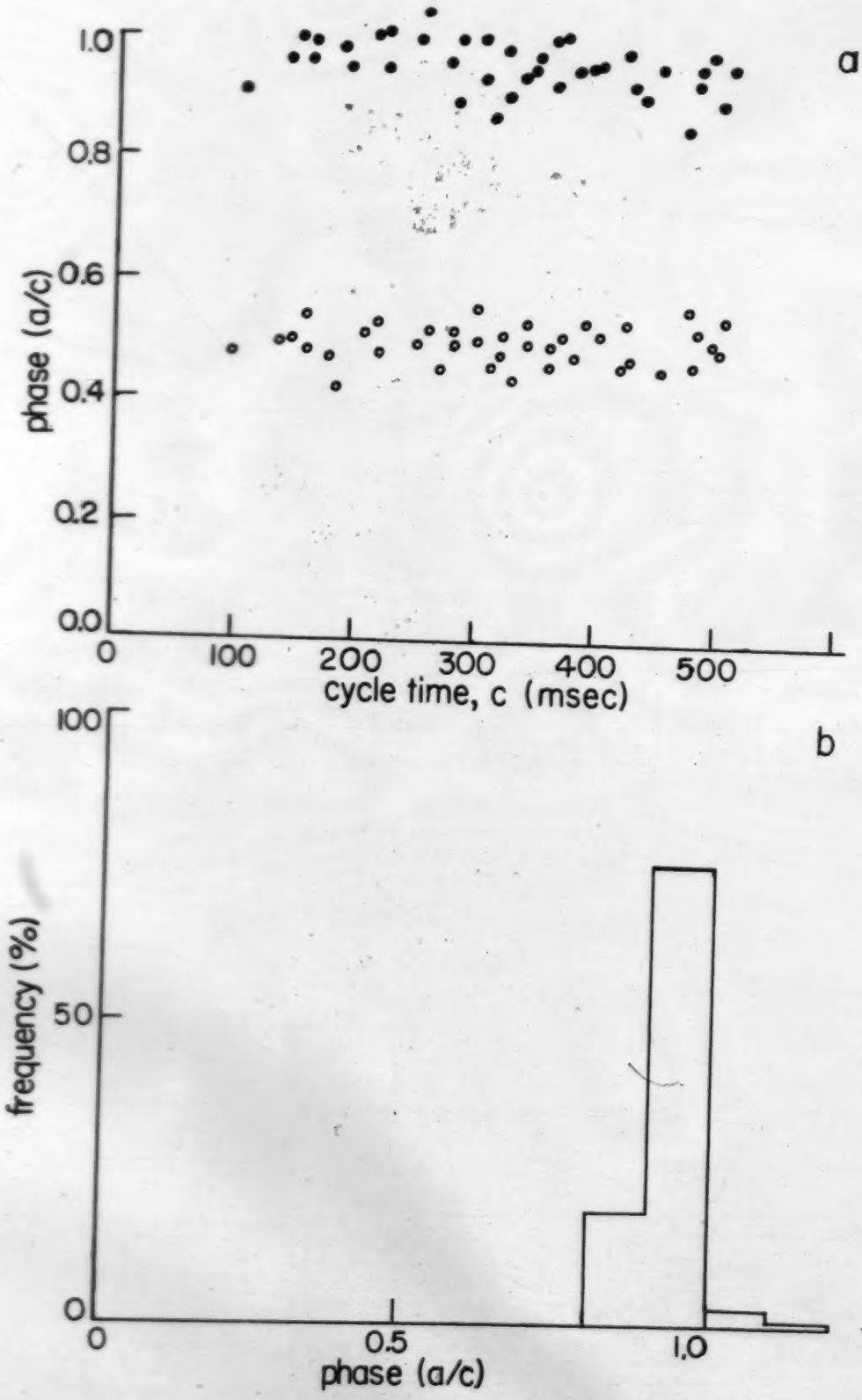


Figure 2. Part a shows the burst activity recorded from the coxal levator muscles (flexors of the femur) of ipsilateral metathoracic (top trace), mesothoracic (middle trace) and prothoracic (bottom trace) legs. Each burst occurred during the protraction phase of leg movement. The two bursts per step cycles of the prothoracic levator muscles correspond to two protractions of the leg during one complete step cycle. The first prothoracic flexor burst was used in determination of phase. Part b illustrated the latencies that were measured in order to calculate the phase of one burst in the cycle of another. Lag time, a, is the time from the beginning of one burst to the beginning of another burst in a different leg. Cycle time, c, is the time from the beginning of one burst to the beginning of the next burst in the same segment. Phase is calculated by dividing lag time by cycle time. The calibration bar represents 250 milliseconds.

Figure 3. Phase relationships for ipsilateral legs are plotted versus cycle time in part a for intact animals walking on a flat surface. The phase of stepping of a prothoracic leg in the cycle of a metathoracic leg is denoted by solid circles (●) and is around 1.0. The phase of stepping of a mesothoracic leg in the cycle of a metathoracic leg is denoted by open circles (0) and is around 0.5. The phase relationships are independent of cycle time. Thus, the phase values can be plotted as a frequency histogram as illustrated in part b for the pro- and metathoracic legs. The data in part b represents phase values calculated from a total of 97 step cycles recorded from six animals.


a
1.0
0.8
0.6
0.4
0.2
0.0
phase (a/c)
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300
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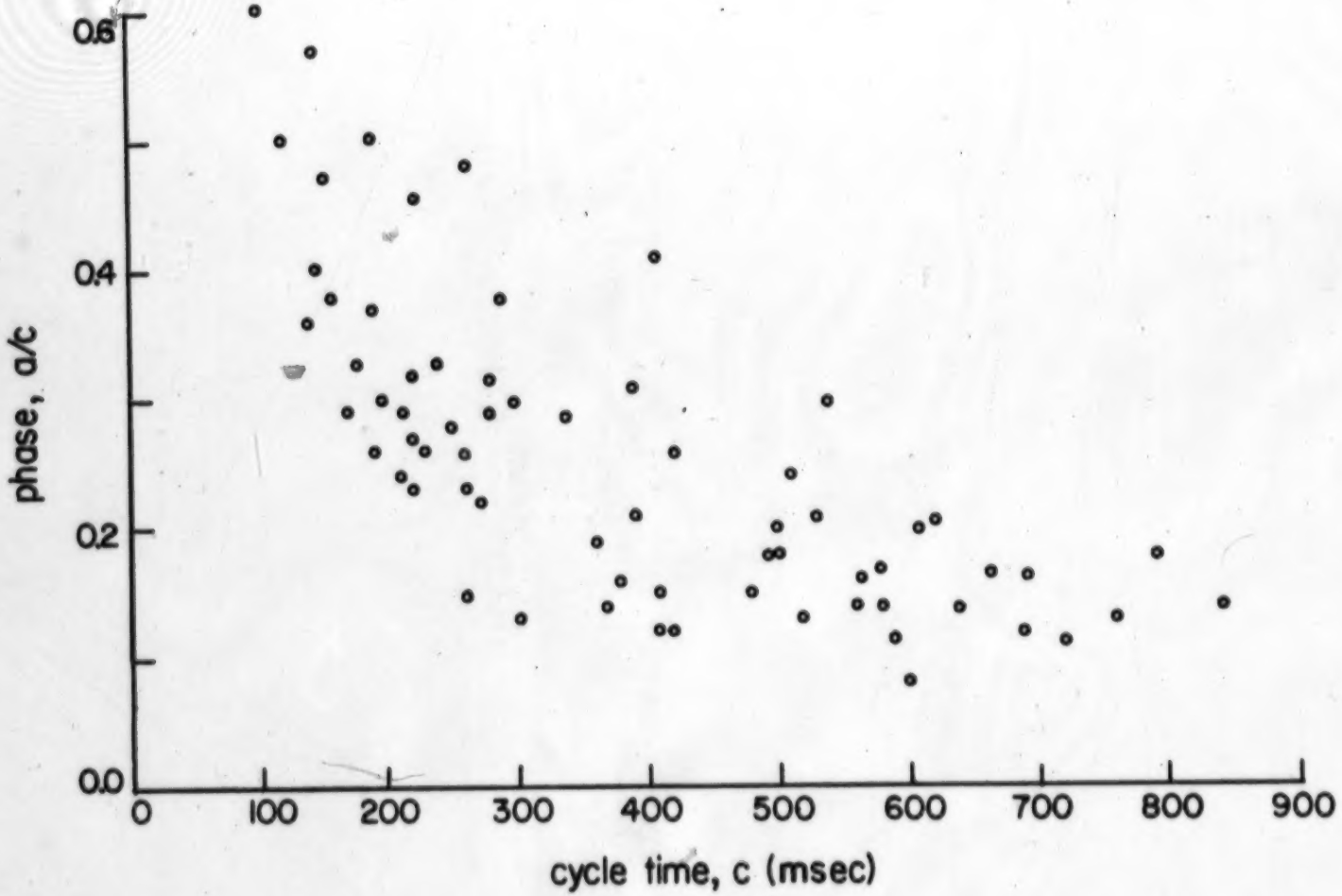
cycle time, c (msec)
b
100
50
0
frequency (%)
0
0.5
1.0
phase (a/c)

by other investigators (Delcomyn, 1971b; Wendler, 1966; von Buddenbrock, 1921), and to examine more closely the central coordinating pathways between ipsilateral flexor burst generators. Also, since one of the purposes of this investigation was to investigate the mechanical linkage of intrasegmental reflexes, the effects of amputation of the mesothoracic legs and restraint of the mesothoracic legs were compared. The change in loading of the remaining four legs was similar in these two procedures; however, as will be discussed later, the change in the stepping pattern between ipsilateral pro- and metathoracic legs was not the same in these two cases.

The most interesting observation from the amputation experiments was that following removal of the mesothoracic legs distal or proximal to the trochanter, there was an immediate change in the stepping pattern of the remaining four legs. In a L2 R2 amputee walking on a flat horizontal surface, the phase relation between ipsilateral pro- and metathoracic legs varied from 0.1 to 0.6 (fig. 4). The phase of protraction of a prothoracic leg in the cycle of the ipsilateral metathoracic leg in these animals depended strongly on walking speed. This was brought about by the fact that the intersegmental delay between stepping of the pro- and metathoracic legs was relatively constant (fig. 5). So as the cycle period increases, the phase of L1 and L3 decreases. In the intact animal, intersegmental delay increases with increasing cycle time, thus producing a constant phase relation over all walking speeds.

The question is then whether the change in stepping pattern exhibited by the amputees was the result of the new mechanical

Figure 4. The phase values determined for the stepping of a prothoracic leg in the cycle of an ipsilateral metathoracic leg for an amputee walking on a flat horizontal surface are plotted versus cycle time. The mesothoracic legs were amputated distal to the trochanter. The phase values vary from 0.1 to 0.6, and are dependent on cycle time. Eight animals were studied in this experiment.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phase, a/c
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200
300
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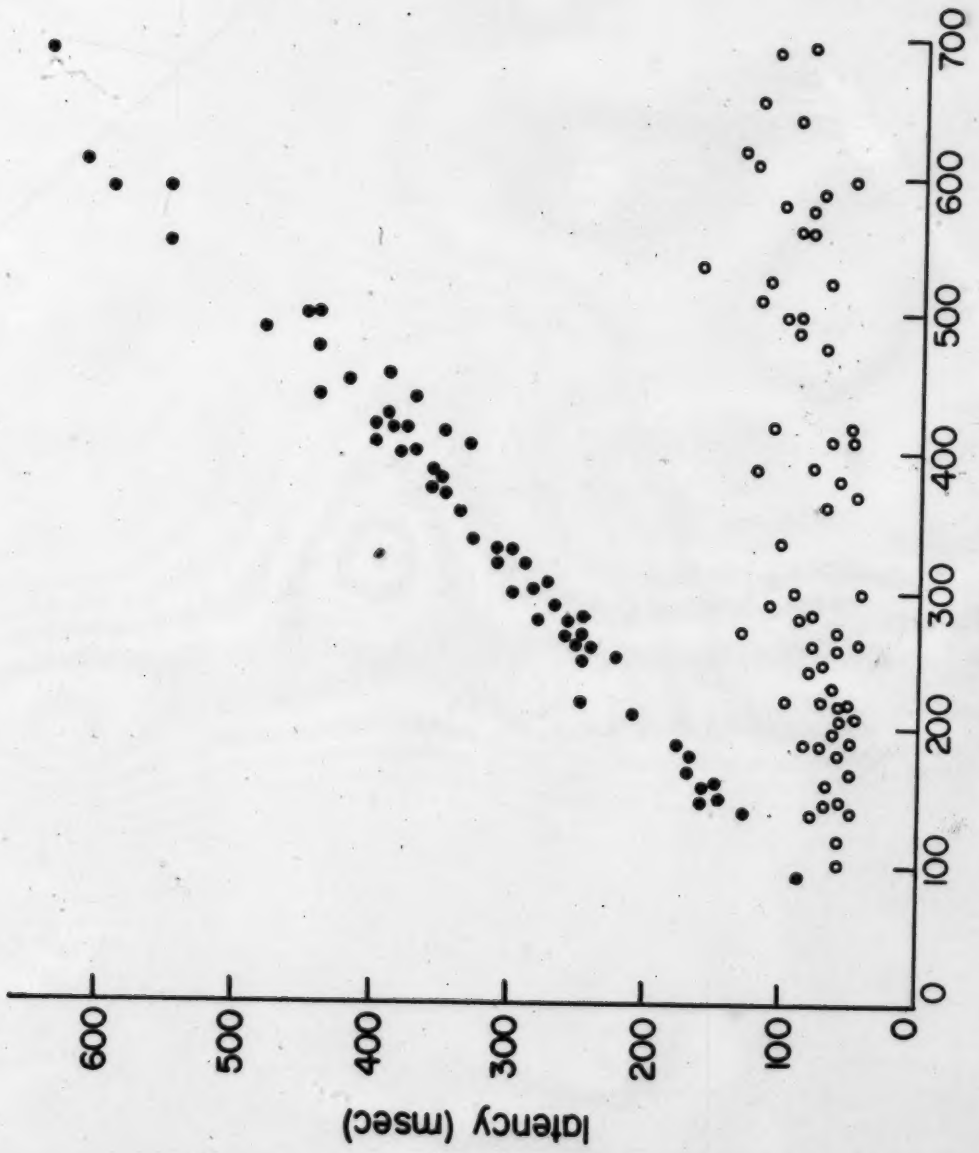
600
700
800
900
cycle time, c (msec)

Figure 5. Latency from the beginning of a metathoracic flexor burst to the beginning of an ipsilateral prothoracic flexor burst is plotted versus cycle time. The solid circles (●) denote the data from intact animals walking on a flat surface. These latencies are dependent on cycle time. The open circles (0) denote the data from mesothoracic amputees walking on a flat surface. These latency values are independent of cycle time.


latency (msec)
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cycle time, c (msec)
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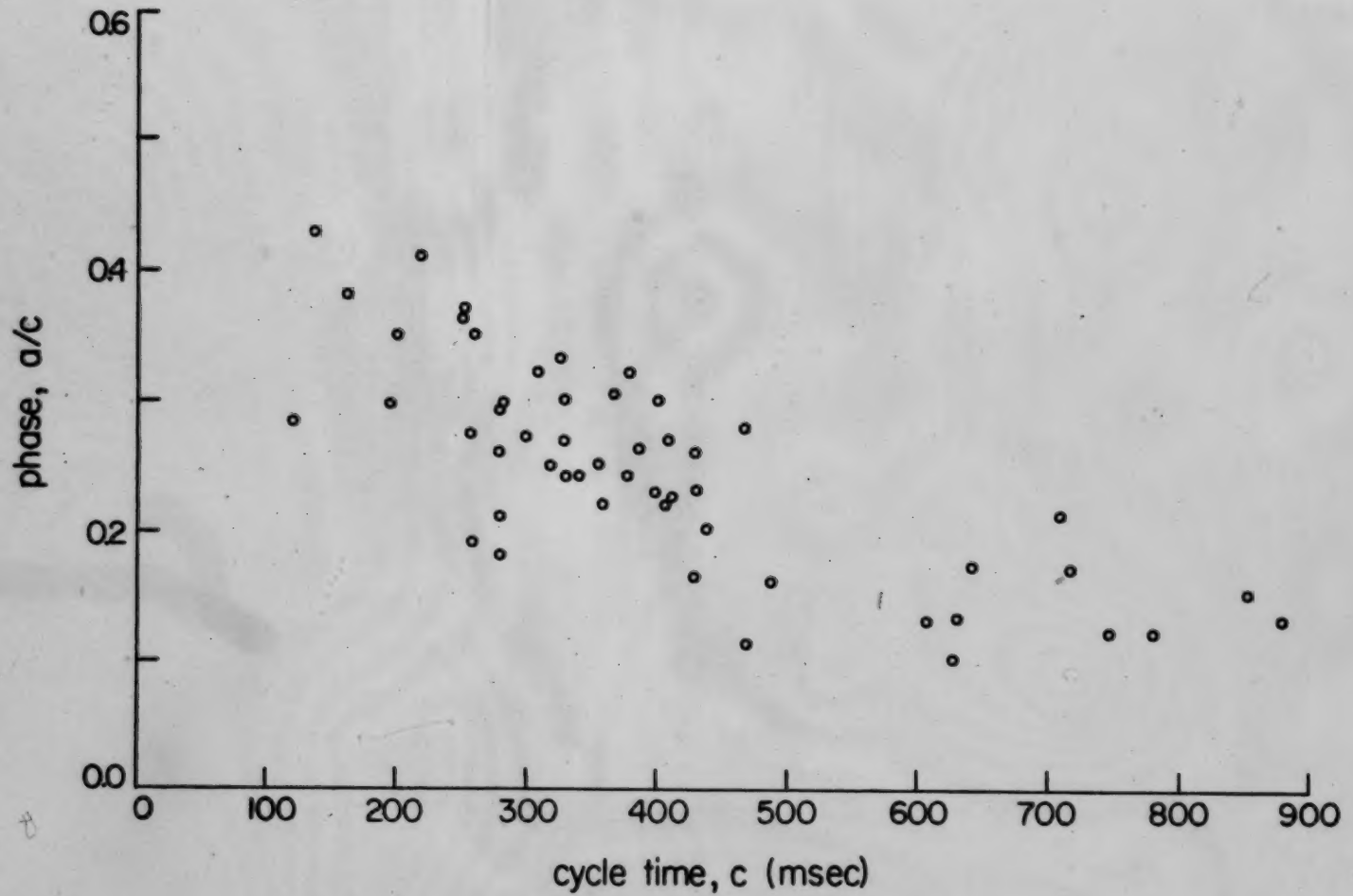
situation or the result of the loss of afferent input from the amputated legs. In order to investigate this problem, the animals were made to walk on a pool of mercury while their bodies were supported. Since the animal's body was supported and held stationary, a step in one leg could not alter the load carried by other legs. Therefore, in this situation, the change in loading of the legs created by amputation of the mesothoracic legs could not have an effect. The phase relations were determined for stepping of the prothoracic leg in the cycle of the metathoracic leg in the resulting stepping movements. It was found that the phase relations were identical to the phase relations found for an amputee walking on a flat surface (fig. 6). One must conclude that the change in stepping pattern observed after amputation of the mesothoracic legs was the result of a loss of sensory input from the missing mesothoracic legs, rather than the result of an altered mechanical condition. This interpretation agrees with previous work (Delcomyn, 1971b; Wendler, 1966; von Buddenbrock, 1921). A model to describe how the lack of sensory input modifies the sequence of stepping in the remaining legs is presented in the discussion.

The change in coordination after amputation of the mesothoracic legs was associated with an altered pattern of burst activity in the mesothoracic flexor motoneurons. After amputation of the leg distal to the trochanter, two flexor bursts occurred in one cycle of metathoracic bursts rather than one (fig. 7a). The first mesothoracic burst began immediately after the cessation of a metathoracic burst. It also appeared that in amputees the prothoracic flexor bursts were

Figure 6. The phase values determined for the stepping of a prothoracic leg in the cycle of an ipsilateral metathoracic leg for amputees walking on a pool of mercury with their weight supported are plotted versus cycle time. The mesothoracic legs were amputated distal to the trochanter. The phase values vary from 0.1 to approximately 0.4 and are dependent on cycle time. Eight animals were used.


0.6
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cycle time, c (msec)

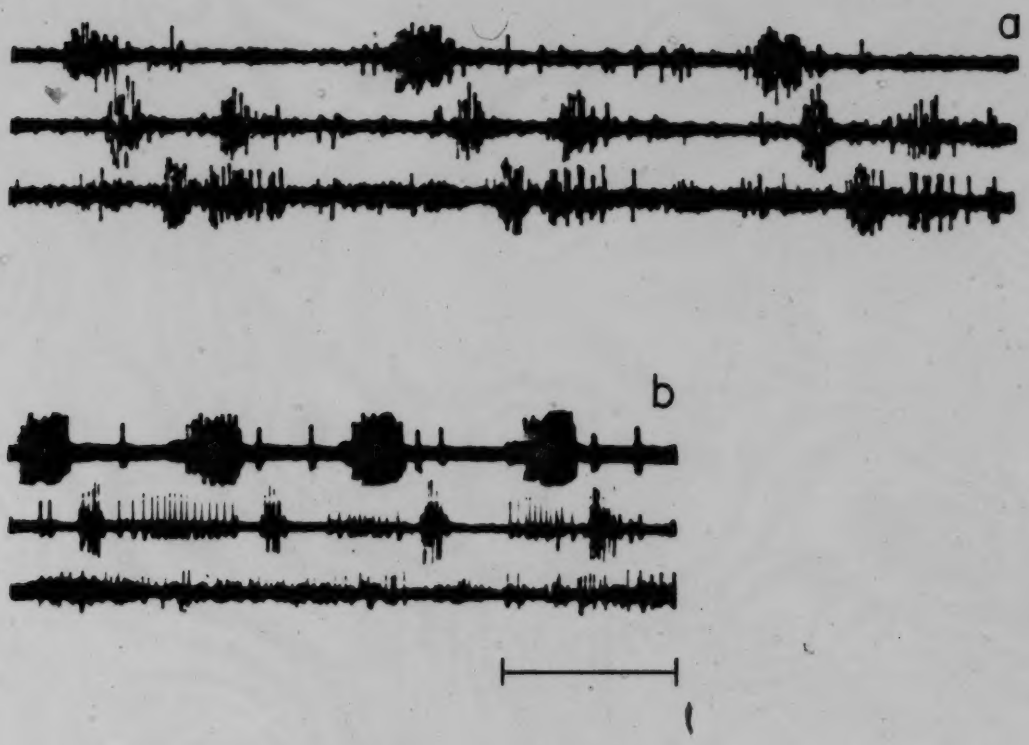

Figure 7. Example of the activity recorded from the coxal levator muscles of ipsilateral legs in amputees walking on a flat surface. Part a is an example of records obtained from animals with their mesothoracic legs amputated distal to the trochanter. It shows the burst activity recorded from metathoracic (top trace), mesothoracic (middle trace) and prothoracic (bottom trace) legs. Part b is an example of records obtained from animals with their mesothoracic legs amputated proximal to the trochanter. It shows the burst activity from metathoracic (top trace), prothoracic (middle trace) and mesothoracic (bottom trace) legs. Note the absence of distinct mesothoracic levator bursts in proximal amputees (part b). The calibration bar represents 250 milliseconds.

initiated soon after the first of the two mesothoracic bursts. In contrast, after amputation proximal to the trochanter no distinct bursts were recorded in the mesothoracic flexors (fig. 7b). Since the flexor burst generators of the pro- and metathoracic segments are postulated to be centrally coupled via the flexor burst generator of the mesothoracic segment, any change in the activity of the mesothoracic flexor burst generator might be expected to alter the relative timing of flexor burst activity in the pro- and metathoracic segments. In the animals in which the legs were amputated distal to the trochanter, the burst activity in the mesothoracic flexors and the phase relation between the pro- and metathoracic legs was drastically different from the intact animal. However, for animals with their legs amputated proximal to the trochanter, no distinct bursts were recorded from the mesothoracic flexors yet the phase relation between the pro- and metathoracic legs was the same as in the case where the legs were amputated distal to the trochanter. Since the mesothoracic flexor burst activity was different while the coordination between the pro- and metathoracic legs was the same, this suggested that activity in the mesothoracic flexor burst generators did not affect the timing of stepping in the pro- and metathoracic legs, i.e., the pro- and metathoracic flexor burst generators are not coupled to one another via the mesothoracic flexor burst generator in this situation. Rather, the pro- and metathoracic flexor burst generators may somehow be 'directly' coupled to one another by a central neural pathway *not* involving the mesothoracic flexor burst generator.

The receptors involved in the behavioral modifications of the

sort described for amputees are probably in the proximal portion of the leg. These receptors include the campaniform sensilla and the hair plate. Amputation of the distal aspects of the appendages did not greatly affect the stepping patterns (Delcomyn, 1971b). Also, when prosthetic limbs were provided for proximally amputated legs, thereby re-establishing their contact with the substrate, normal stepping was obtained (Hughes, 1957; Wendler, 1966).

Restraint of the Mesothoracic Legs

Walking on a flat surface. Another method of altering stepping patterns and mesothoracic levator burst activity is to restrain legs so that they may not participate in locomotion. Restraint of the mesothoracic legs by tying them in a flexed position against the thorax had a great effect on coordination of stepping between the pro- and metathoracic legs. The phase relations between the pro- and metathoracic legs was variable but centered around 0.5 to 0.6 instead of normal 1.0. The phase was independent of cycle time and therefore could be plotted as frequency histogram (fig. 8).

The new 0.5 phase relation exhibited by an animal with its mesothoracic legs restrained would be expected to give a mechanically stable stepping pattern. Since restraining the mesothoracic legs alters the mechanical situation, it is quite possible that receptors in the pro- and metathoracic legs which are sensitive to the mechanical state of the leg, e.g., loading, would detect the altered mechanical condition and influence the flexor burst generator accordingly. Leg receptors, namely the campaniform sensilla which detect the mechanical

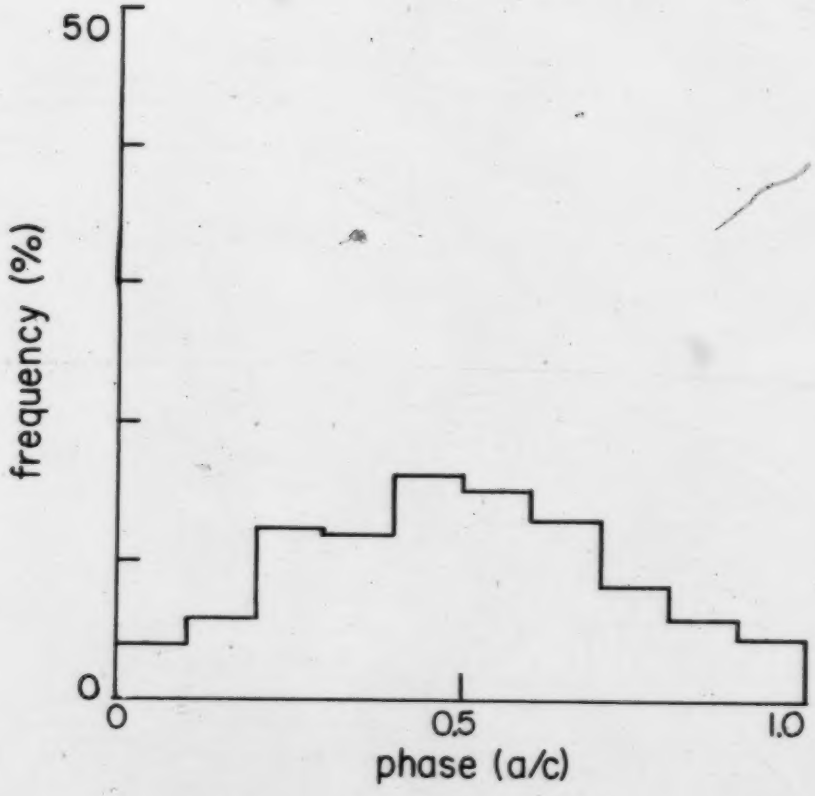

Figure 8. The phase values determined for the stepping of a prothoracic leg in the cycle of an ipsilateral metathoracic leg for animals walking on a flat surface with their mesothoracic legs restrained. A total of 356 phase values were calculated but only the step cycles in which no mesothoracic levator bursts occurred were graphed (total = 235). Ten animals were used.

parameter of load, have been described (Pringle, 1938; Spinola & Chapman, 1975). However, the legs also have a variety of other receptors: chordotonal organs, hair plates and sensory hairs (Guthrie & Tindall, 1968). Therefore, it seems unlikely that the campaniform sensilla are the only receptors involved in detecting the new mechanical situation brought about by the restraint of the mesothoracic legs.

Recordings were made of the coxal flexor and extensor muscles of the restrained mesothoracic legs. There was almost continuous firing of the excitatory extensor motoneuron, D_s (fig. 9a) and a two-thirds reduction in the occurrence of flexor motoneuron bursts during walking (fig. 9b). No rhythmic movements of the restrained mesothoracic legs were observed. Also, in the crab, binding of the middle legs resulted in a cessation of rhythmic movements of the bound legs (Evoy & Fourtner, 1973). When flexor bursts in the restrained mesothoracic legs did occur, they always followed the flexor bursts of the metathoracic leg and usually occurred singly. The bursts in the prothoracic leg always followed the bursts in the mesothoracic leg (fig. 9b). The inhibition of the mesothoracic flexor burst generator and the increased firing of D_s by restraint of the mesothoracic legs is thought to be a result of input from the campaniform sensilla. Previously it had been shown that sensory input from the campaniform sensilla inhibits flexor motoneuron activity, i.e., inhibits the flexor burst generator and excites extensor motoneuron activity (Pearson & Iles, 1973; Pringle, 1940). Strain of the cuticle has been shown to be an effective stimulus for the campaniform sensilla (Pringle, 1938) and it is likely that tying the mesothoracic legs to the thorax

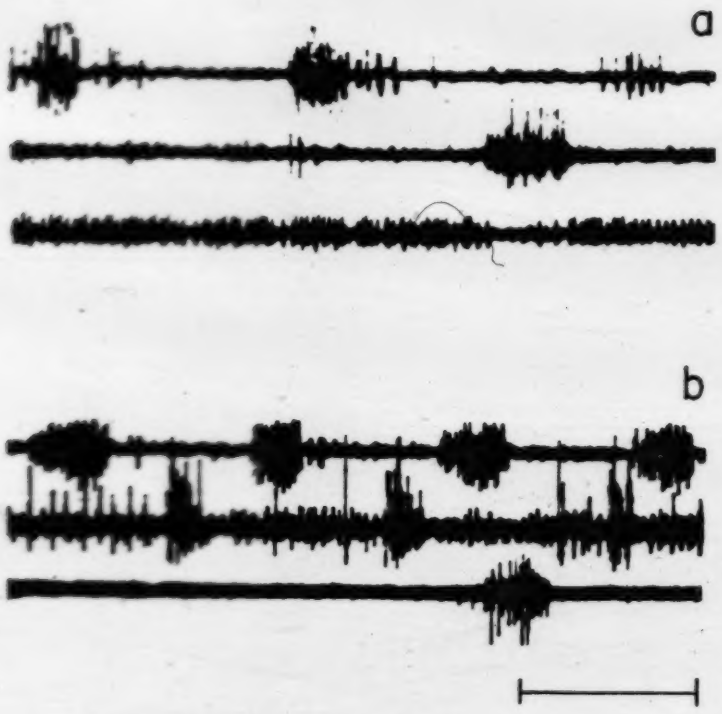

Figure 9. Example of the activity recorded from ipsilateral legs in animals walking on a flat surface with their mesothoracic legs restrained. Part a shows the burst activity recorded from metathoracic (top trace) and mesothoracic (middle trace) coxal levator muscles. Note the cessation of mesothoracic depressor muscle activity (bottom trace) when the mesothoracic flexor burst occurred. Part b shows the burst activity from the metathoracic (top trace), prothoracic (middle trace) and mesothoracic (bottom trace) legs. Bursts in the mesothoracic leg occurred 121 times out of the 356 metathoracic step cycles analyzed. The calibration bar represents 250 milliseconds.

strained the cuticle of the trochanter. So in the situation where the mesothoracic legs were restrained, input from the campaniform sensilla was probably responsible for the partial inhibition of the mesothoracic flexor burst generator. The fact that the flexor burst generator was not completely inhibited could be the result of depression of the inhibitory activity of the campaniform sensilla.

Ablation of the campaniform sensilla. To test the hyopthesis that the campaniform sensilla were responsible for the inhibition of the mesothoracic flexor burst generator, the campaniform sensilla of the trochanter (groups 1 to 5) of the restrained legs were destroyed as described in the methods section. After ablation of the receptors, mesothoracic flexor bursts occurred in 85% of the step cycles as compared to 34% when the campaniform sensilla were intact. However, there are campaniform sensilla at each sensory spine of the leg (Chapman, 1965) and these campaniform sensilla or other leg receptors could have been responsible for the remaining inhibition of the flexor burst generator as these receptors were not destroyed. In particular, since the trochanteral hair plates are thought to inhibit the flexor burst generator (Pearson, Wong and Fourtner, 1976) these receptors may be partially responsible for the inhibition of the flexor burst generator when the legs were restrained. No attempt was made to destroy these receptors and determine if they were partially responsible for the inhibition. However, on the basis of the ablation experiments, the campaniform sensilla were shown to be mainly responsible for the inhibition of the flexor burst generator when the mesothoracic legs were restrained.

The data plotted in the frequency histogram for an animal walking on a smooth surface with its mesothoracic legs restrained (fig. 8) represents only those step cycles in which no mesothoracic flexor bursts occurred. The step cycles in which flexor bursts occurred were analyzed separately (fig. 10a). Also, the phases of L1 in L3 were determined for the animals which had the campaniform sensilla destroyed on the restrained mesothoracic legs (fig. 10b). The frequency histograms for all these experiments were similar with the mean phase around 0.6. Therefore, regardless of whether the mesothoracic flexor burst generator was or was not inhibited, the same stepping patterns were exhibited.

The data for animals with mesothoracic legs restrained and the campaniform sensilla destroyed on the restrained legs was also analyzed with respect to the phase relationships between L3 and L2 and between L2 and L1. It should be noted that the L1 flexor burst always followed the L2 flexor burst. The latency between the L2 and L1 bursts was variable but less so than the latency between the L2 and L2 bursts. For steps in which the L2 flexor burst occurred, the phase of L2 bursts in the cycle of L2 burst showed a fairly strong coupling with the phase around 0.7 to 0.9 (fig. 11a). The coupling between L3 and L2 bursts was weaker, i.e., the phase was more variable with the mean around 0.3 and 0.4 (fig. 11b). From these results, it seems that restraining the mesothoracic legs brings about a greater uncoupling of L3 and L2 than of L2 and L1. These results also apply to animals with the campaniform sensilla intact on the restrained legs.

Figure 10. Part a is the frequency histogram of the phase values determined for stepping of a prothoracic leg in the cycle of an ipsilateral metathoracic leg for animals walking on a flat surface with their mesothoracic legs restrained. Only those step cycles in which a mesothoracic flexor burst occurred were used in determination of these phase values. Ten animals were used and the phase values were obtained from a total of 121 step cycles. Part b shows the data obtained from animals in which the campaniform sensilla on the mesothoracic legs were destroyed before the legs were restrained. Five animals were studied and a total of 149 step cycles were examined.

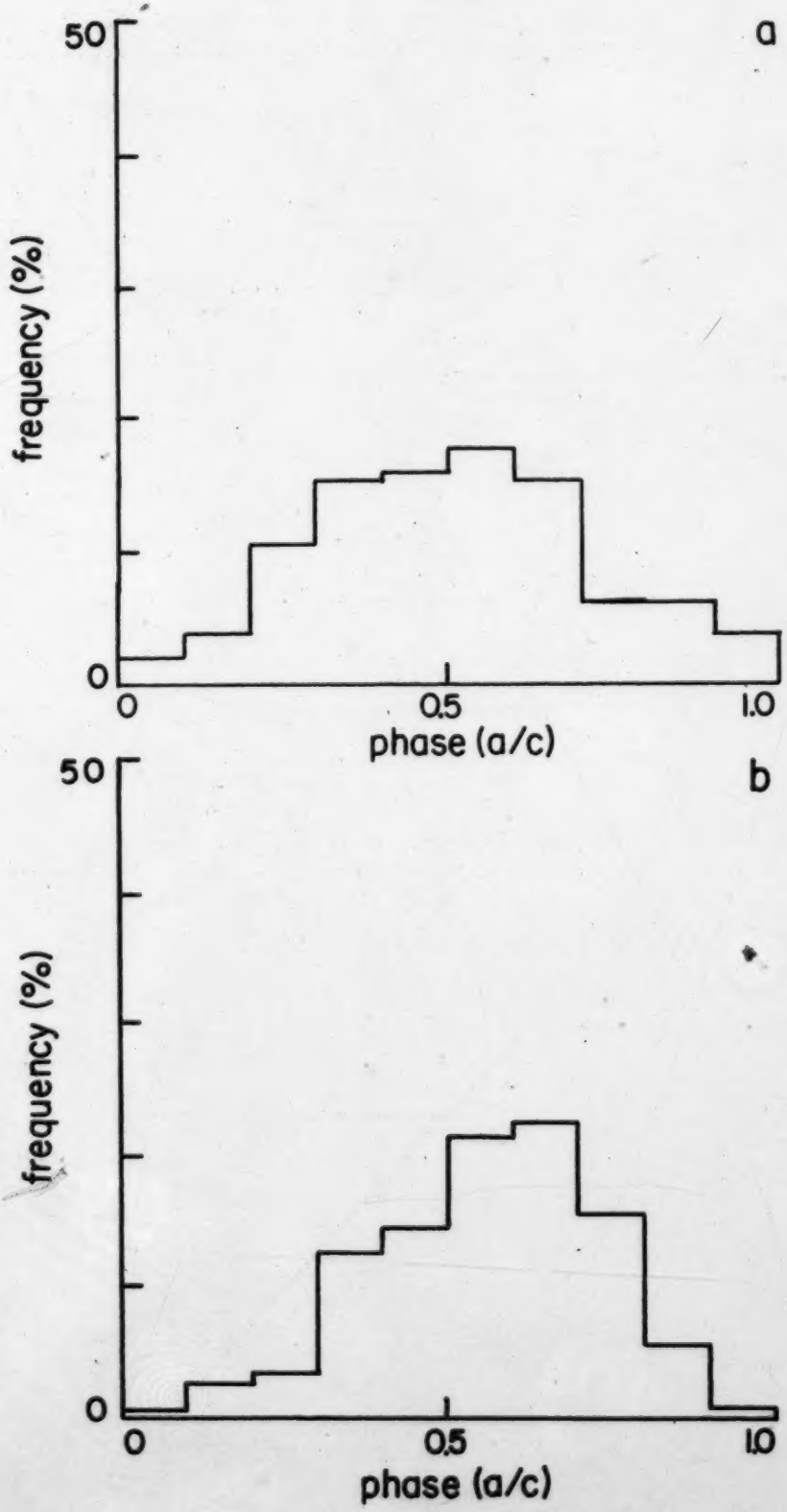
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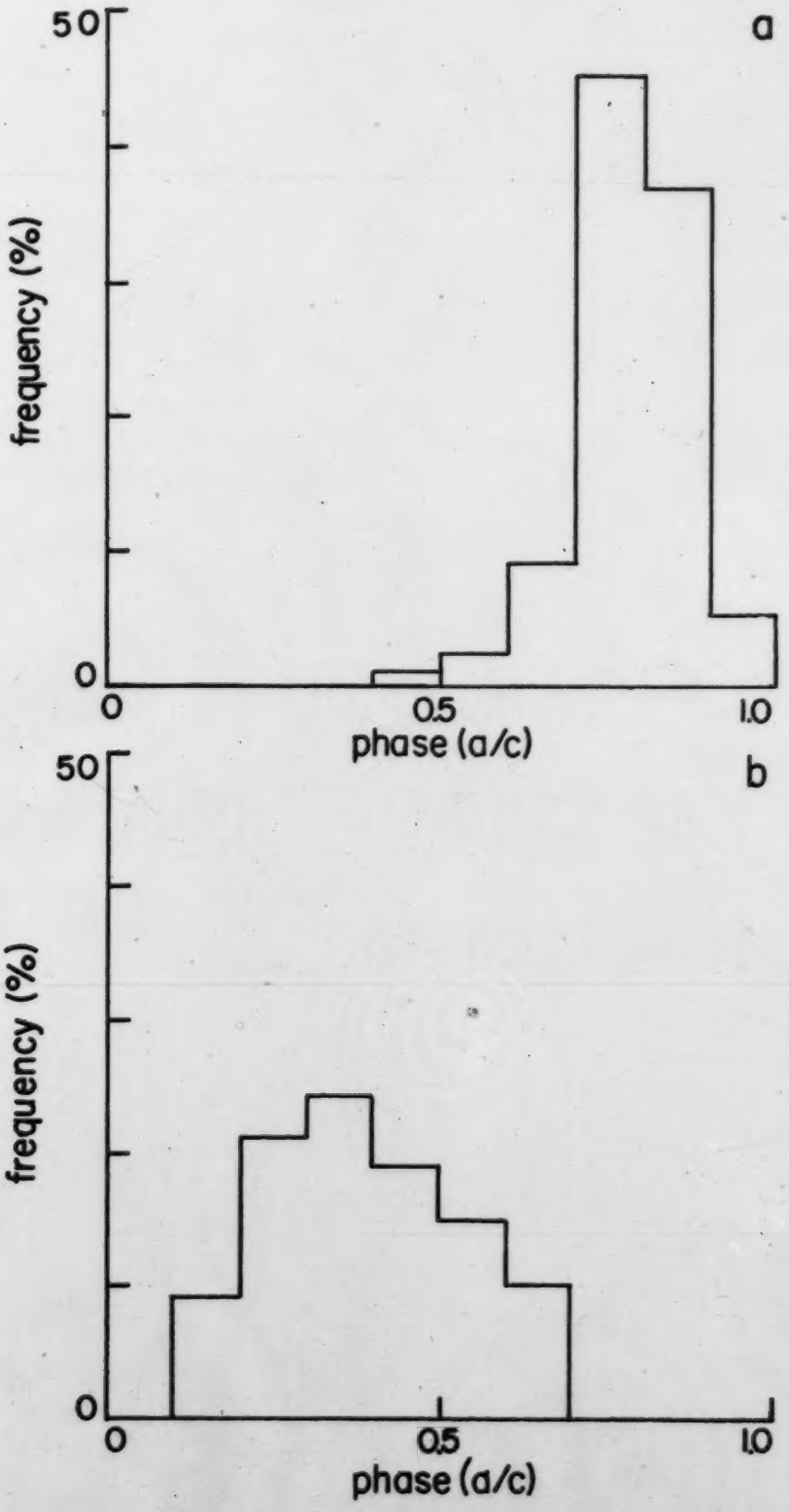
Figure 11. Part a is a frequency histogram of the phase values for stepping of a mesothoracic leg in the cycle of an ipsilateral prothoracic leg for animals walking on a flat surface with their mesothoracic legs restrained. Part b shows the phase values for stepping of a mesothoracic leg in the cycle of an ipsilateral metathoracic leg. The campaniform sensilla of the mesothoracic legs were destroyed in these animals to increase the frequency of occurrence of the mesothoracic flexor bursts. Five animals were used and a total of 149 step cycles examined.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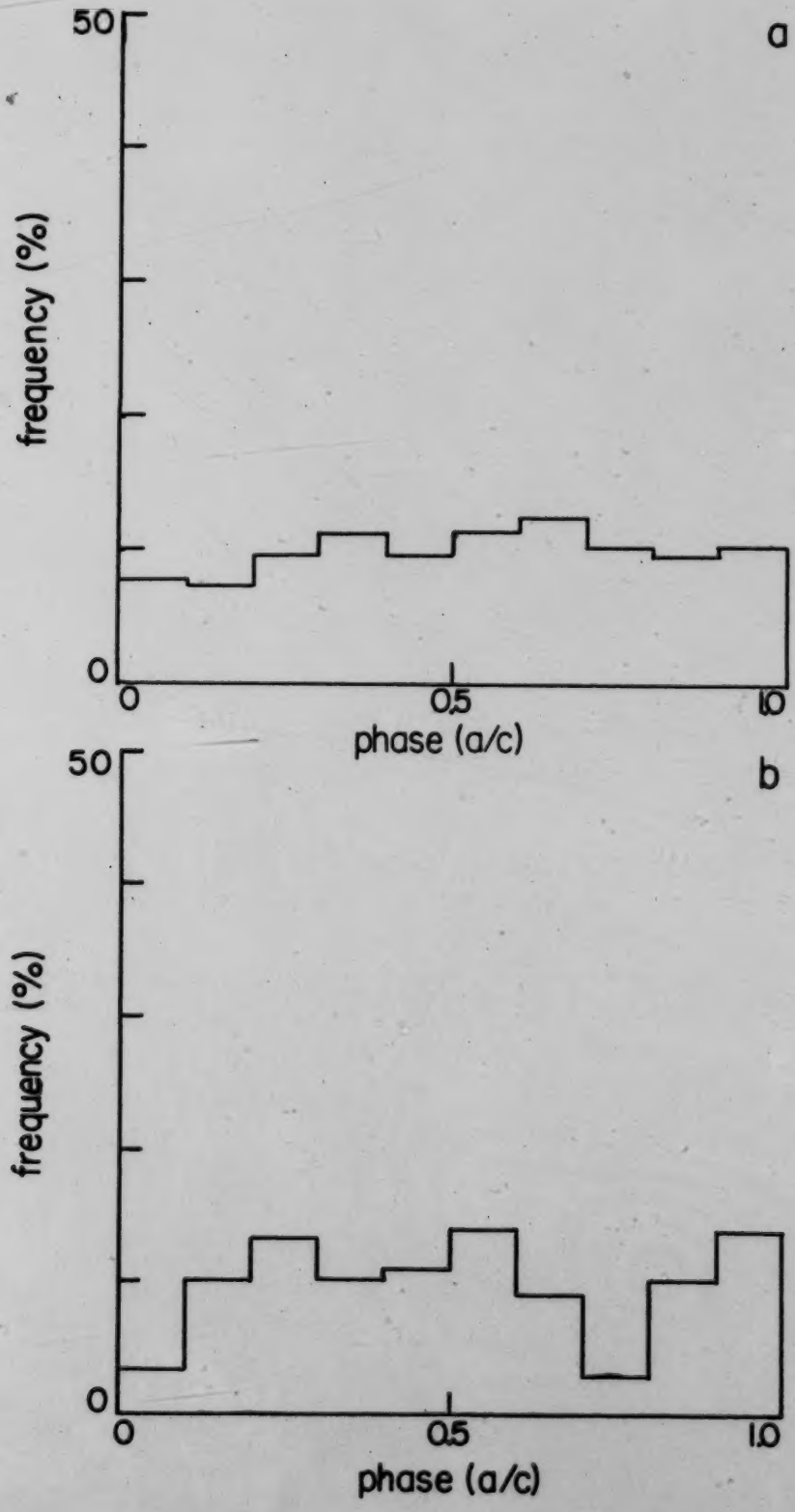
Walking on mercury. On the basis of the data obtained from experiments in which the mesothoracic legs were restrained, it seems possible that a mechanical linkage of intrasegmental reflexes could function to coordinate stepping. The phase of stepping of a prothoracic leg in the cycle of the metathoracic leg was around 0.5 for an animal with its mesothoracic legs restrained. This represents the most mechanically stable phase relationship. To test the idea that the movement of one leg actually affects the timing of stepping movements of the other legs, the animals were made to walk upon a pool of mercury with their weight supported while their mesothoracic legs were restrained. In this situation, a stepping movement in one leg could not alter the load carried by another leg. Flexor burst activity in ipsilateral left legs was recorded. Interestingly, no burst activity was recorded from the mesothoracic flexor muscles; even when the trochanteral campaniform sensilla of these legs were destroyed.

Subsequent analysis of the phase of stepping of a prothoracic leg in the cycle of an ipsilateral metathoracic leg showed that the phase relation was completely variable, i.e., the flexor bursts in the pro- and metathoracic segments exhibited all phase relationships (fig. 12a). In animals with the campaniform sensilla of the restrained legs were destroyed, the phase was also variable (fig. 12b) and no L2 flexor bursts occurred. Thus, the flexor burst generators of the pro- and metathoracic segments were completely uncoupled when the mesothoracic legs were restrained and the animal's weight was supported. Presumeably, the central neural pathways responsible for intersegmental coordination were inoperative and the mechanical linkage of intra-

Figure 12. Part a is a frequency histogram of the phase values for stepping of a prothoracic leg in the cycle of an ipsilateral metathoracic leg. The animals had their mesothoracic legs restrained and walked over a pool of mercury while their weight was supported. Nine animals were studied and a total of 238 step cycles examined. Part b is the data from animals in which the campaniform sensilla on the mesothoracic legs were destroyed. Four animals were used and a total of 89 steps were examined. No mesothoracic flexor bursts occurred when the animals walked on mercury.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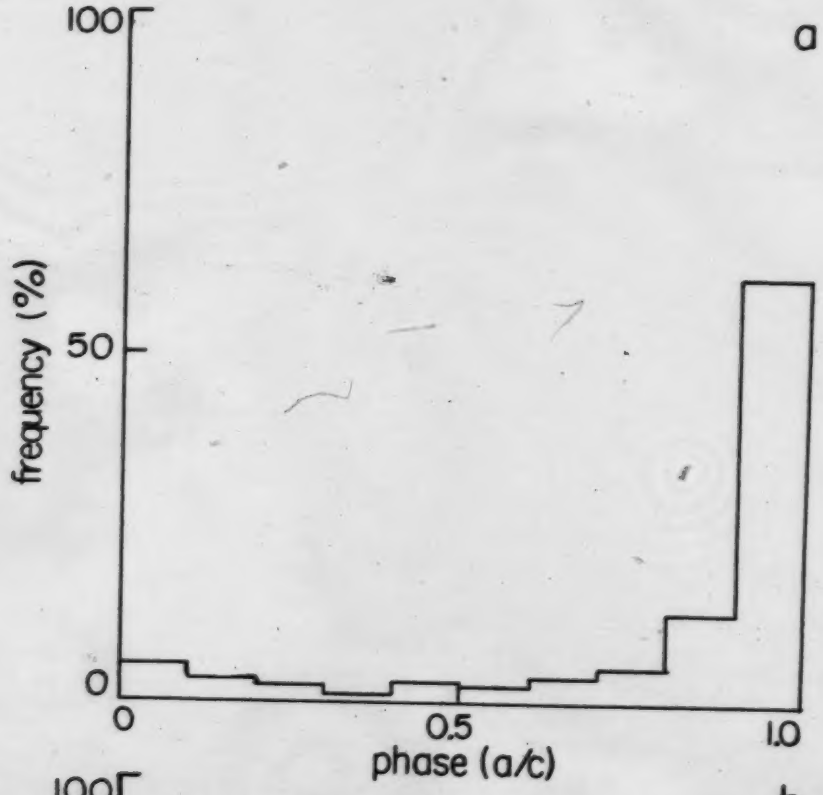
phase (a/c)

segmental reflexes was broken. So in the situation in which the mesothoracic legs were restrained and the animal was walking on a flat surface, it was the mechanical linkage of intrasegmental reflexes that was responsible for the 0.5 phase relationship exhibited by the pro- and metathoracic legs.

Control experiments were done in which animals were made to walk on mercury with or without their weight supported. For intact animals walking on mercury and supporting their own weight, the phase of L1 in L3 was close to 1.0 which is the phase relation seen in intact animals walking on a smooth horizontal surface (fig. 13a). The same phase relation was also exhibited for animals with their weight supported (fig. 13b). Some variation in the phase relation was seen but the mean phase was not significantly different between animals supporting their own weight while walking on a flat surface or on mercury. The variability in the phase relation came about as a result of the prothoracic leg occasionally stepping twice curing one cycle of the metathoracic leg. The prothoracic leg appeared to be making 'searching' movements. Also, for animals walking on mercury with their mesothoracic legs restrained and supporting their own weight, the phase histogram was similar to that determined for similar animals walking on a flat surface (fig. 13c). Therefore, the substrate does not in itself greatly affect the timing of the stepping movements between different legs.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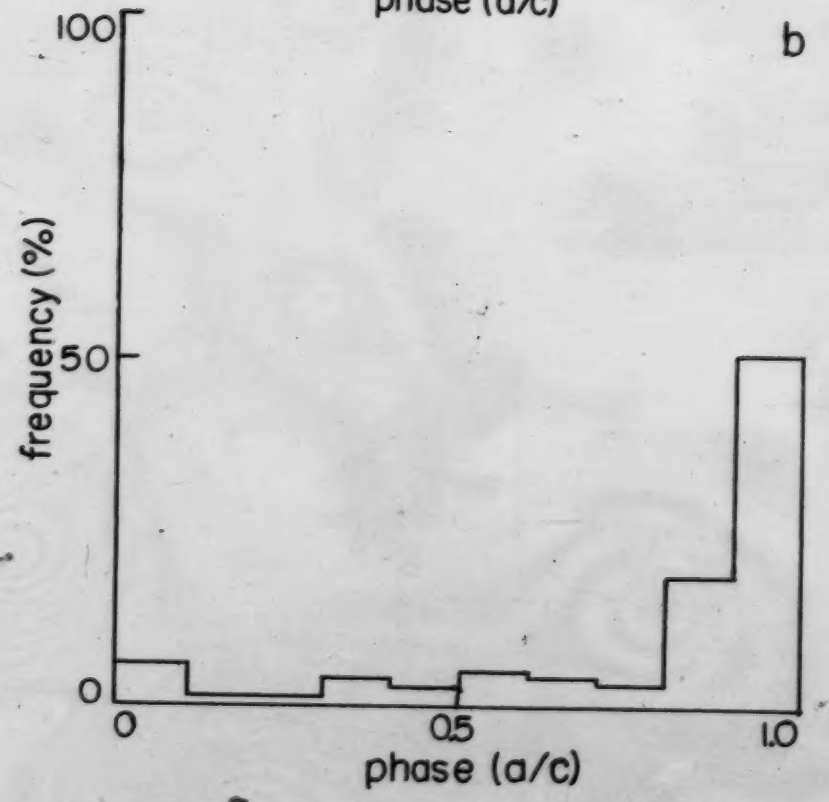
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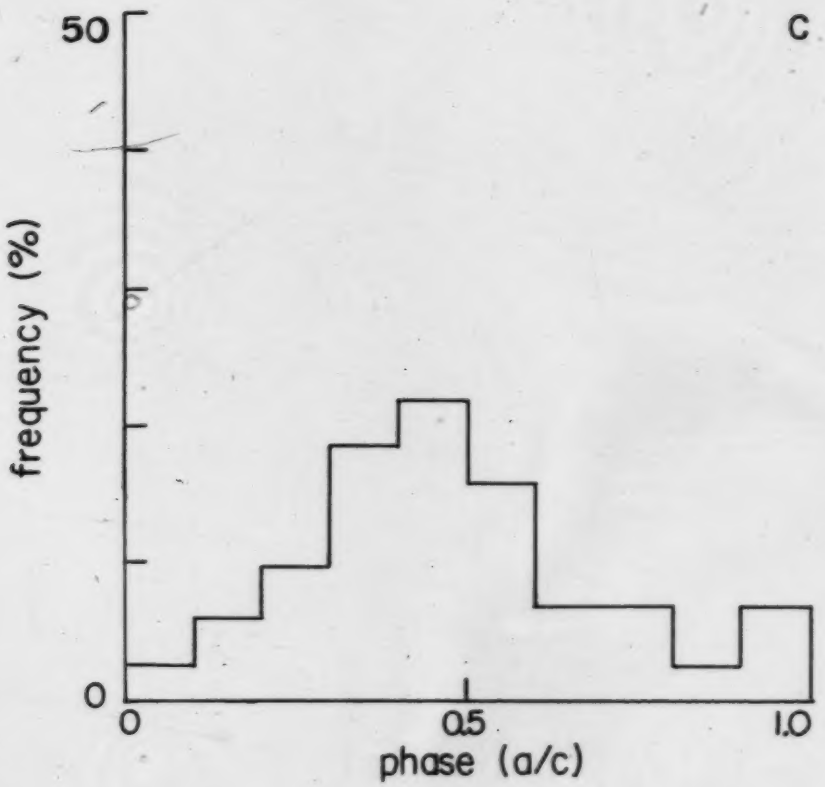

Figure 13. The frequency histograms show the phase relationships between a prothoracic leg and an ipsilateral metathoracic leg for animals walking on mercury. Part a is the data for normal intact animals supporting their own weight. Part b is data for normal intact animals with their weight supported. Three animals were used and 104 step cycles were examined for parts a and b. Part c is data for animals supporting their own weight with their mesothoracic legs restrained. Three animals were studied and 151 step cycles examined.

DISCUSSION

Intersegmental Coordination: Central Neural Pathways

The intent of this study was to examine the mechanisms responsible for coordinating the stepping movements of the legs, in particular the pro- and metathoracic legs during walking. Previous work has demonstrated central inhibitory coupling between the mesothoracic and metathoracic flexor burst generators and has led to the suggestion that the pro- and metathoracic flexor burst generators are coupled to one another via the mesothoracic flexor burst generators (Pearson & Iles, 1973). Evidence for central coordinating mechanisms has also been obtained for the crayfish swimmeret system (Stein, 1971) and the leech swimming system (Kristan & Calabrese, 1976).

To test the hypothesis that the pro- and metathoacic flexor burst generators are coupled via the mesothoracic flexor burst generator procedures were employed which altered the burst activity in the mesothoracic flexor burst generator (Table I). Table I shows that the phase of stepping of a prothoracic leg in the cycle of an ipsilateral metathoracic leg was not obviously related to the pattern of burst activity in the mesothoracic segment. However, it should be noted that the activity of the mesothoracic flexor burst generator was not recorded directly. Only the activity in the flexor muscles was recorded. Thus it is possible that while no bursts were recorded from the muscles in certain situations, there may have been subthreshold activity in the flexor burst generator itself. The effect of such subthreshold activity, if it occurred, on adjacent flexor burst

TABLE I

BURST ACTIVITY IN THE MESOTHORACIC COXAL LEVATOR MUSCLES DURING VARIOUS EXPERIMENTAL CONDITIONS

Experimental Condition	Phase of stepping of a prothoracic leg in the cycle of an ipsilateral metathoracic leg	Mesothoracic Levator Burst Activity
1. Normal intact animal walking on a flat surface	1.0; dependent on cycle time	single burst
2. Amputation of mesothoracic legs distal to trochanter; walking on a flat surface	0.1 to 0.6; dependent on cycle time	double bursts
3. Amputation of mesothoracic legs proximal to trochanter; walking on a flat surface	0.1 to 0.6; dependent on cycle time	no distinct bursts
4. Amputation of mesothoracic legs distal to trochanter; walking on mercury while weight is supported	0.1 to 0.4; dependent on cycle time	double bursts
5. Mesothoracic legs restrained; walking on flat surface	mode$\sim$ 0.5; independent of cycle time	bursts only occurred in$\sim$ 34% of step cycles
6. Mesothoracic legs restrained; campaniform sensilla of the mesothoracic leg destroyed; walking on flat surface	Mode$\sim$ 0.6; independent of cycle time	bursts occurred in$\sim$ 84% of step cycles
7. Mesothoracic legs restrained; walking on mercury while weight is supported	Completely variable; independent of cycle time	no distinct bursts

TABLE I (continued)

Experimental Condition	Phase of stepping of a prothoracic leg in the cycle of an ipsilateral metathoracic leg	Mesothoracic Levator Burst Activity
8. Mesothoracic legs restrained; campaniform sensilla destroyed on mesothoracic legs; walking on mercury while weight is supported	completely variable; independent of cycle time	no distinct bursts

generator could not be determined.

Amputations of the mesothoracic legs distal or proximal to the trochanter brought about a radical change in the coordination of stepping of the pro- and metathoracic legs (fig. 4) and a change in the flexor burst pattern in the mesothoracic segment when the animal walked on a flat surface (fig. 7). In this situation, the phase relationship between the pro- and metathoracic legs varied from 0.1 to 0.6 and was dependent upon the duration of the step cycle. After amputation of the legs distal to the trochanter, the flexor burst generator of the mesothoracic segment produced two bursts during each step cycle of the metathoracic leg instead of the 'normal' one. By contrast, when the mesothoracic legs were amputated proximal to the trochanter, no distinct bursts were produced in the mesothoracic segment. However, the stepping pattern of the pro- and metathoracic legs was the same, i.e., the phase varied from 0.1 to 0.6 and was dependent on cycle time. Thus, the pattern of burst activity in the mesothoracic flexor motoneurons was not related to the coordination of the pro- and metathoracic legs. This suggests that the pro- and metathoracic flexor burst generators may also be coupled by a direct pathway not involving the mesothoracic flexor burst generator (see fig. 14). In the model, this pathway is shown as an inhibitory pathway via a coordinating neuron. To explain the burst pattern exhibited by amputees, this direct pathway is thought to receive inhibitory input from proximal mesothoracic leg receptors. These receptors could include the campaniform sensilla, hair plates or possibly chordotonal organs. The campaniform sensilla and hairplate have been shown to

inhibit the flexor burst generator (Pearson & Iles, 1973); Pearson, Wong, Fourtner, 1976, respectively). As yet very little is known about the chordotonal organs. The flexor burst generator postulated to receive tonic excitatory input from proximal leg receptors (Pearson, 1972) is as yet unidentified. The effects of amputation can be explained as follows. Amputation of the mesothoracic legs, either distal or proximal, to the trochanter removes the inhibitory influence on the direct central pathway from the meta- to the prothoracic flexor burst generator. Thus when a metathoracic flexor burst occurs, the prothoracic flexor burst generator is prevented from bursting by inhibitory input from coordinating interneurons driven by the metathoracic flexor burst generator. In distal amputees, the mesothoracic flexor burst generator can then burst after the metathoracic burst has terminated by post-inhibitory rebound. After the mesothoracic flexor burst is terminated, the prothoracic flexor burst generator can burst as it no longer receives inhibitory input from the meta- or mesothoracic flexor burst generators. A second mesothoracic flexor burst occurs as a result of a termination of inhibitory input from the prothoracic flexor burst generator by post-inhibitory rebound. This explains the stepping pattern exhibited by amputees, i.e., the constant delay between the meta- and prothoracic segments which is independent of cycle time.

The result that no distince mesothoracic flexor bursts are recorded after amputation proximally could be explained as a reduction of excitatory input from proximal receptors onto the flexor burst generator as a result of the higher level amputation.

It was determined that the new stepping pattern observed after amputation was the result of loss of afferent input from the amputated legs and not brought about by the new mechanical situation created by amputation. This was shown by placing the animals in a situation where a stepping movement of one leg could not affect the loading or the position of the other legs (walking on mercury while the body was supported). Under these conditions, the pro- and metathoracic legs exhibited the same phase relationships (fig. 6) as determined for amputees walking on a flat surface carrying their own weight. In other words, the mechanical linkage of intrasegmental reflexes was not responsible for the observed stepping patterns. The new stepping pattern is explained by the suggested model (fig. 14) in which there is a direct inhibitory pathway from the metathoracic flexor burst generator onto the prothoracic flexor burst generator.

Intersegmental Coordination: Mechanical Linkage of Intrasegmental Reflexes

Results obtained in this study provided support for the hypothesis that a mechanical linkage of intrasegmental reflexes can serve as a coordinating mechanism during walking. In other words, the movement state of one leg can affect the timing of stepping in other legs as a result of an alteration in activity in intrasegmental reflex pathways in other legs. This was shown by comparing the phase relationship between stepping of ipsilateral pro- and metathoracic legs for animals with their mesothoracic legs restrained walking on: 1) a flat surface supporting their own weight and 2) mercury with their weight supported. In the first situation, the phase histogram

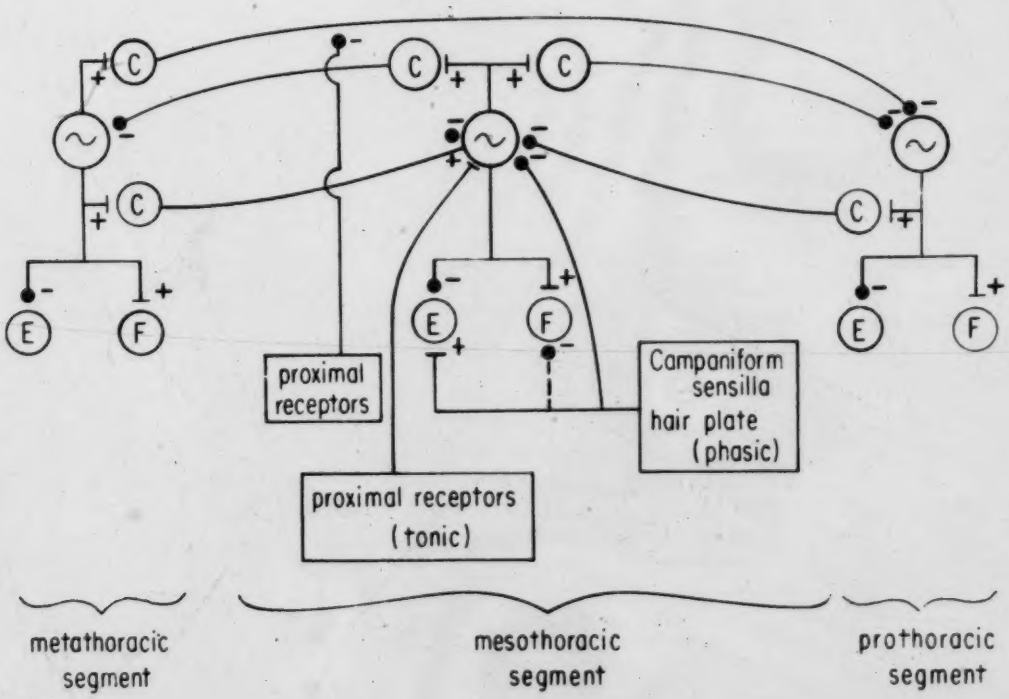

Figure 14. Model showing the postulated central pathways between the flexor burst generators of three ipsilateral segments. Intrasegmental reflex pathways are diagrammed for the mesothoracic segment only. The hair plate also excites the flexor motoneurons (dotted line). Proximal receptors refer to those receptors involved in the effects observed after amputation and tying of the mesothoracic legs. Further explanation in the text. E, extensor motoneurons; F, flexor motomeurons; C, coordinationg neurons; ∿, flexor burst generator; -, inhibitory connection; +, excitatory connection.

showed a peak around 0.5 (fig. 8). Similar results have been obtained for walking in the crab (Evoy & Fourtner, 1973). Binding of the middle legs of the crab in maximal levation resulted in a change in the phase relation of stepping amongst the other legs. With the middle legs restrained, the phase of stepping of a foreleg in the cycle of the ipsilateral hindleg was around 0.5. The phase histogram for the crab was less variable than that seen for the cockroach. The variability in the phase relation came about as a result of the prothoracic leg occasionally stepping twice during one step cycle of the metathoracic leg. The prothoracic leg appeared to be making 'searching' movements. Cruse (1977) has suggested that the prothoracic legs also function as feelers in the stick insect. In the cockroach, the prothoracic legs may not only function as walking legs but as feelers as well.

In the second situation where the animal's weight was supported, the phase histogram showed complete variability (fig. 12a). The same phase relationships were observed in the animals with the campaniform sensilla of the restrained legs destroyed (fig. 12b). The fact that the phase relationship was completely variable when the animals' weight was supported, suggested that it was the mechanical linkage of intrasegmental reflexes that was responsible for the 0.5 phase relationship exhibited by animals walking on a flat surface with their mesothoracic legs restrained. Alternate stepping of the ipsilateral pro- and metathoracic legs would be expected to be the most mechanically stable walking pattern in this situation. Thus, it is clear that the mechanical linkage of intrasegmental reflexes can

serve as a coordinating mechanism. Figure 15 illustrates the operation of this coordinating mechanism. Tying up the mesothoracic legs inhibits the mesothoracic flexor burst generators and the direct pathway from the meta- to the prothoracic flexor burst generators. Coordination of stepping movements of the pro- and metathoracic legs is then brought about by the mechanical linkage of intrasegmental reflexes.

The sensory receptors involved in the mechanical linkage of intrasegmental reflexes probably involve several types of proprioceptors. An important type may be the campaniform sensilla of the trochanter. Ablation of the trochanteral campaniform sensilla on the restrained mesothoracic legs increased the occurrence of mesothoracic flexor bursts to approximately 84% of the step cycles from 34% when the sensilla were intact. Since the campaniform sensilla inhibit the flexor burst generator (Pearson & Iles, 1974) it is likely that the decrease is the result of this inhibitory influence of campaniform sensilla. Other receptors, such as the trochanteral hair plates may also be involved. These receptors give information about leg position as they are excited by flexion of the femur (Wong & Pearson, 1976). They have been shown to excite the depressor motoneurons and to inhibit the flexor burst generator (Pearson, Wong and Fourtner, 1976).

Since the campaniform sensilla appear to be involved in load detection and have an inhibitory effect on the flexor burst generators of each leg, they could be responsible, to a large degree for ensuring that a leg does not step when it is loaded. Thus it is quite possible that these receptors in the pro- and metathoracic legs were involved

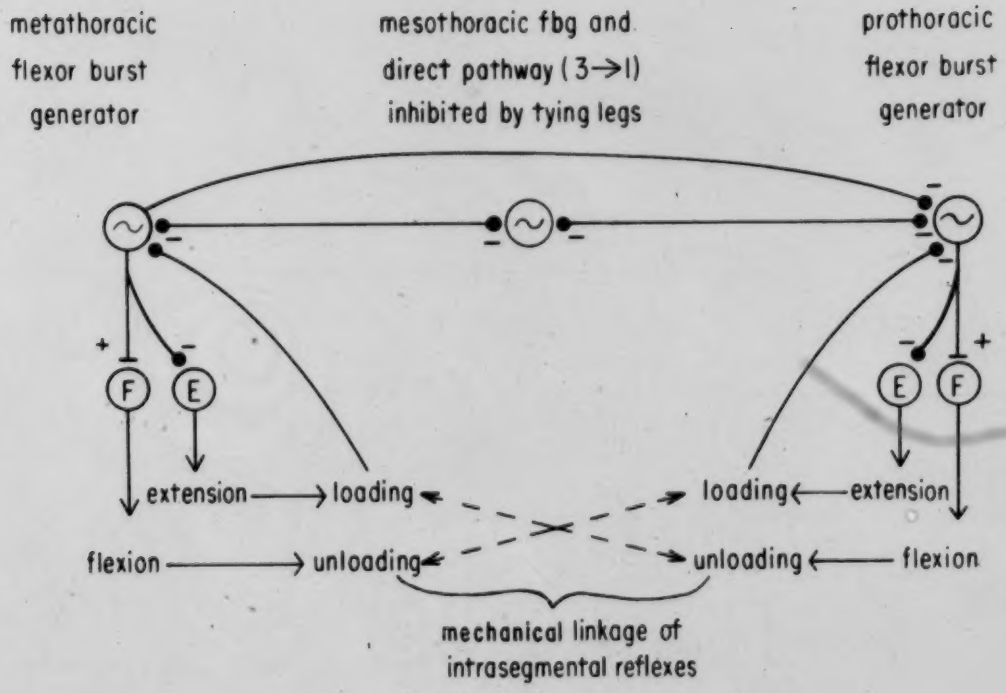

Figure 15. Schematic diagram illustrating the mechanical linkage of intrasegmental reflexes. E, extensor motoneurons; F, flexor motoneurons; ∿, flexor burst generator; -, inhibitory connection; +, excitatory connection.

in detecting the altered mechanical situation created by tying the mesothoracic legs to the thorax and played a role in ensuring that the ipsilateral pro- and metathoracic legs did not step at the same time.

The role of mechanical linkage of intrasegmental reflexes in normal walking has not been assessed. It is unlikely that when the animal is walking on a smooth surface that this mechanism is important. It probably is important when the animal walks over uneven terrain (Pearson & Iles, 1973). While an animal is walking slowly, the load carried by each leg will vary from moment to moment. If the load carried by a leg decreases below a certain level, then the inhibition to the flexor burst generator of that leg will be insufficient to prevent the initiation of a flexor burst. Thus the leg will step and the load carried by that leg will be distributed amongst the other legs. Hence, the probability that the other legs will step decreases because of an increase in inhibition of their flexor burst generator. Another leg may begin stepping during the step of the first leg since the load carried by the second leg may decrease as a result of change in position of the animal as it moves forward. However, the mutual inhibitory coupling between adjacent flexor burst generators ensures that the second stepping leg is not adjacent to the first. When the stepping leg makes contact with the ground the load carried by the other legs, particularly adjacent legs, will decrease and these may step immediately or shortly after depending on their exact position. The existence of inhibitory reflex pathways to the flexor burst generator in each hemiganglion provides

a mechanism for allowing the animal to traverse uneven and unpredictable terrain in a stable manner.

FUTURE WORK

Two questions of intersegmental coordination need further investigation: 1) what is the role and exact nature of the central neural pathways?; 2) what signals are responsible for cycle-dependent intersegmental delay?

One approach to studying the nature of the central neural coordinating pathways would be to record from interganglionic interneurons involved in intersegmental coordination. Two procedures could be used to electrophysiologically identify coordinating interneurons. One would be to record extracellularly from fibers dissected from the thoracic connectives and the second would be to record either intracellularly or extracellularly with microelectrodes from the processes of interneurons with axons in the connectives. It would be necessary to demonstrate that activity in these interneurons was: 1) coupled to activity of one flexor burst generator and 2) could affect the timing of the initiation of a burst in another flexor burst generator. It would also be necessary to show that these interneurons were not sensory fibers. This could be done by either recording from deafferented preparations or by determining the structure of the cells. One technique for visualizing the structure of the neurons is by the cobalt technique (Pitman, Tweedle & Cohen, 1972).

In order to fully understand intersegmental coordination, it will be necessary to determine what signals are used to bring about cycle-dependent intersegmental delays. In light of the results from mesothoracic amputees, it appears that sensory signals are utilized

to some extent to produce cycle-dependent intersegmental delays. It would be interesting to determine which sensory receptors are involved, how the information is utilized and to what extent the system relies on this sensory information. Central pathways may also be involved, however, these pathways would have to be more fully described before any assessment could be made of their contribution to the generation of cycle-dependent intersegmental delays.

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